International Journal for Multidisciplinary Research (IJFMR)
E-ISSN : 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Effects of lead on some oxidative stress of the Catfish, *Clarias batrachus*

Dr. Ravindra Singh

Associate Professor, Department of Zoology, K.B.P.G College Mirzapur, U.P., India

Abstract

This study aimed to determine the effects of sub-lethal concentrations of lead (PbCl2) on some oxidative stress in the catfish *Clarias batrachus*. Fishes were captured from Ramgarh Lake, Gorakhpur. They were acclimatized and fed with commercial fish diet for one month before starting the experiment. Two experimental groups were exposed to two sublethal concentrations (10% and 30% of LC_{50}) of lead chloride, fishes from different experimental groups were sacrificed after 60 days of exposure. Oxidative stress enzymes such as GSH, CAT, SOD, and MDA were estimated. Group treated with Pb10 % showed significantly lower concentrations of GSH. On the other hand, Pb 30% group showed a significantly higher concentration of CAT, MDA and SOD**.**

Keywords: Lead, oxidative, stress, Catfish, *Clarias batrachus*.

INTRODUCTION

Heavy metals generally have different effects on fishes depending on the type of heavy metal, its concentration in water and the period of exposure (Boscher *et al.*, 2010). Many studies have shown that bioaccumulation of heavy metals affects their growth rate and the quality of their meat, beside to its significant effect on their physiological and chemical status (Soegianto *et al.*, 2010). Some heavy metals are essential in the environment system such as copper, chromium, and iron, while others such as cadmium and lead are not necessary and are toxic even in low concentrations (Rubio *et al.*, 2016).

Lead is a non - essential metal; however, it is easily absorbed into the body through ingestion or inhalation and undergoes bioaccumulation, the risk of exposure to the effects of lead is very high (Łuszczek-Trojnar *et al.*, 2014), however, exposure to this metal causes changes in metabolism as well as many biochemical and physiological effects (Szebedinszky *et al.*, 2001). Fishes convert the foreign compounds enzymatically into less harmful compounds, this reaction is often the most important processxenobiotic biotransformation (Persch *et al.*, 2017).

The catfish, *Clarias batrachus*, has a great nutritional and economic importance because it is preferable food for a lot of Indian. Being one of the most common freshwater fish, it's a good animal model for investigating various environmental pollutants (Darwin *et al.*, 2017). This species is resistant to environmental stress and can adapt to different types of food in the environment and its availability at night or day (Qu *et al.*, 2014).

Exposure to lead in the environment leads to its accumulation in fish tissue, later, many serious disorders appear in the metabolism, behavior change, abnormalities in movement, and obvious effects on blood (Gabriel *et al.*, 2004). Studies on the effect of lead on *C. batrachus* showed that it caused a reduction in oxidative stress and CAT was significantly higher compared to the control group (Loveline *et al.*, 2018).

Exposure of the Indian catfish *C.batrachus* to Pb $(NO_3)_2$ caused reduction in GSH, SOD, CAT and MDA compared to control (Saliu and Bawa-Allah, 2012).

The present study aimed to measure the changes of oxidative stress enzymes (malondialdehyde. MDA; Catalase, CAT; glutathione, GSH and superoxide dismutase, SOD) in catfish after exposure to different doses of lead chloride.

MATERIALS AND METHODS

Determination of LC_{50}

One hundred and seventy-two catfish, *Clarias batrachus* (mean body weight =150±250g) were obtained from Ramgarh lake, Gorakhpur. Fishes, apparently healthy, were transported immediately to the lab and acclimatized for two weeks in glass aquaria (40* 35* 70 cm) with a capacity of 60 L and were fed on a basal fish diet. After acclimation,100 fishes were divided into ten groups (10 fishes/ group) for determination of LC_{50}, each group was exposed to a different concentrations of $PbCl_2$ (100 mg/l, 150 mg/l, 200 mg/l, 250 mg/l, 300 mg/l, 350 mg/l, 400 mg/l, 450 mg/l,) at constant temperature 25ºC. Fishes were kept under observation for 96 hours and numbers of dead fishes were recorded daily. The LC_{50} was statistically determined by using dose-effect analysis using XLSTAT software.

Experimental fishes model:

After determination of LC_{50}, 72 fishes were divided randomly into equal three groups with 8 fishes in all aquaria, each group was represented by triplets (24 fishes for each treatment). These groups are;

1- Control group,
2- Fish exposed to Pb 10% of LC_{50} for 60 days
3- Fish exposed to Pb 30% of LC_{50} for 60 days

Test Compound

Lead as $PbCl_2$ (molecular weight 278.10) manufactured by Oxford Lab Chem, India.

Blood sampling

Fishes fasted for 24 hours before sampling, blood samples were obtained from the caudal vein using 3 ml syringe within less than 3 minutes to minimize handling stress. The collected blood samples were divided into two tubes. Anticoagulant free samples were used for serum preparation by centrifugation for 20 minutes at 1207 g within one hour of sampling. The sera samples were used for determination of MDA, CAT and GSH, while SOD was determined in RBCs lysate later on the same day with commercial kits. Absorbance values of samples and standards were measured using a UV spectrophotometer.

Statistical analysis

Data were expressed as mean ± SEM of different treated groups compared to control ones. Normal distribution of all parameters was tested. The results were analyzed using oneway analysis of variance (ANOVA) followed by Tukey (HSD) test to compare groups with each other and Dennett two-sided test for comparisons with the control group. $P<0.05$ was considered significant. All statistical analyses were performed using XLSTAT program.

RESULTS AND DISCUSSION

As shown in Table (1), GSH concentration was significantly lower in Pb 10% group compared to control group, while CAT activity was significantly higher in Pb10% and Pb30% group compared to control group. Lipid peroxide product (Malondialdehyde, MDA) was significantly higher in Pb30% group compared to control group, while superoxide

dismutase (SOD) activity was significantly lower in Pb30% group compared to control group.

Table 1: Means± SEM of Lipid peroxidation and oxidative system of catfish, *Clarias batrachus* treated with 10% , 30% Pb of LC_{50} for 60 days.

Parameter	Groups			
	Control	Pb 10% of LC_{50}	Pb 30% of LC_{50}	P
GSH(mg/dl)	15.69± 1.59 [a]	12.21± 0.94 [b]	16.35± 1.72 [a]	0.001
CAT (U/l)	86.98± 29.19 [a]	189.8± 51.53 [ab]	338.8± 49.11 [b]	0.002
MDA(nmol/ml)	24.99± 5.49[a]	39.23± 11.64[ab]	64.41± 7.91[b]	0.009
SOD (U/gm)	144.13± 6.61 [a]	221.8± 46.79 [ab]	264.5± 32..8 [b]	0.007

Values different superscript letters within each row are significantly different (analysis of variance, $P<0.05$).

In the current study, GSH concentration was significantly lower in Pb 10% respectively, compared to the control group. Similarly, GSH level, in the current study, decreased significantly in Pb 10% LC_{50}-exposed group. In line to this finding, Catfish *Clarias batrachus* showed decreased levels of GSH after exposure to 100 µg/l, 300 µg/l and 500 µg/l of lead nitrate (Osman *et al.*, 2007). In another study, GSH level was reduced after 28 days exposure to 5.15, 0.52, 0.052 mg/L of $Pb(NO_3)_2$ compared to the controls (Saliu and Bawa-Allah, 2012). The decreased GSH levels in the present study could account for the marked lipid peroxidation tables (1). Glutathione (GSH) serves to protect the cell against oxidative damage as it conjugates with compounds of exogenous and endogenous origin (Liu *et al.*, 2009). Oxidative stress may be initiated by a decline in the antioxidative defense system, GSH loss in fishes reflects the ability of metals in oxidizing sulfhydryl group resulting in inactivation of oxidative enzymes by free radicals (Nwani *et al.*, 2015) and accumulation of reactive oxygen species (ROS) (Osman *et al.*, 2007; Wu *et al.*, 2011). CAT activity increased in 30% of 96 hrs LC_{50} value - exposed groups compared to control group. Some investigators have suggested that severe oxidative stress may suppress the activity of antioxidant defense enzymes due to oxidative damage and a loss of the compensatory mechanisms (Atli *et al.*, 2006; Liu *et al.*, 2006). Similar results on *Clarias batrachus*, showed that lead increased CAT activity after 28 days exposure to different concentration (28, 43 and 57 mg/L) of lead nitrate (Loveline *et al.*, 2018). This increase in CAT activity is due to the degradation of H_2O_2, a potent oxidant at high cellular concentration is affected by CAT due to its induction against increased oxidative stress. MDA, is used as an effective biomarker of toxic pollutants in fish exposed to lead, MDA level, in the current study, increased in Pb30% group compared to the control group. Similarity Maiti *et al.* (2010) described elevated MDA levels in the catfish *Clarias batrachus* following a 60-day exposure to lead, MDA increased in common carp (*Cyprinus carpio*) after exposed to (50 µg/L) of lead for a period of 30 days (Shafiq-ur-Rehman, 2003),

the production of MDA is a marker of lipid peroxidation results from the decomposition of polyunsaturated fatty acid due to oxidative stress (Alfanie *et al.*, 2015).

SOD activity increased significantly in Pb30% compared to the control group. Similar results were found in *Clarias batrachus* where SOD activity increased after exposure to 0.1 , 0.01 and 0.001 of 96hrLC_{50} of $Pb(NO_3)_2$ (Saliu and Bawa-Allah, 2012) , these pattern indicate the activation of protective mechanisms required for removing O2- radical in RBCs
(Zikić *et al.*, 2001).

REFERENCES

1. Alfanie, I.; Muhyi, R. and Suhartono, E. (2015). Effect of Heavy Metal on Malondialdehyde and Advanced Oxidation Protein Products Cencentration A Focus on Arsenic, Cadmium, and Mercury. J. Med.and Bioengineer, 4:
2. Atli, G.; Alptekin, Ö.; Tükel, S. and Canli, M. (2006). Response of catalase activity to Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in five tissues of freshwater fish *Oreochromis niloticus*. Comp. Biochem. Physiol. Part C: Toxic. & Pharm., 143: 218-224.
3. Boscher, A.; Gobert ,S.; Guignard, C.; Ziebel, J.; L'Hoste, L.; Gutleb, A.C.; Cauchie, H.M.; Hoffmann, L. and Schmidt, G. (2010). Chemical contaminants in fish species from rivers in the North of Luxembourg: Potential impact on the Eurasian otter (*Lutra lutra*). Chemosphere, 78: 7--792.
4. Darwin, D., Sarbaini, S., Purwanto, S., Dhiauddin, F., Ilham, M., Fazil, A., 2017. Wastewater Treatment for Catfish (*Clarias batrachus*) Culture by Using Anaerobic Process. Agritech 37, 462-470.
5. Gabriel, U.; Ezeri, G. and Opabunmi, O. (2004) .Influence of sex, source, health status and acclimation on the haematology of *Clarias batrachus* (Burch, 1822). Afr. J. Biotech.,
6. Liu, J., Qu, W., Kadiiska, M.B., 2009. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicology and applied pharmacology 238, 209-214.
7. Loveline, O.C.; Samuel, P.O.; Arimoro, F.; Ayanwale, A.; Auta, Y. and Muhammed, A. (2018). Effects of lead nitrate on catalase production levels in post juvenile *Clarias batrachus* (Burchell, 1822). Int. J. Fish. Aquacul., 10, 1-7.
8. Łuszczek-Trojnar, E.; Drąg-Kozak, E.; Szczerbik, P.; Socha, M. and Popek, W. (2014). Effect of long-term dietary lead exposure on some maturation and reproductive parameters of a female Prussian carp (*Carassius gibelio* B.). Environ. Sci. Poll. Res., 21: 2465-2478.
9. Maiti, A.K.; Saha, N.C. and Paul, G. (2010). Effect of lead on oxidative stress, Na+ K+ ATPase activity and mitochondrial electron transport chain activity of the brain of *Clarias batrachus* L. Bull. Environ. Contam. Toxic., 84: 672-676.
10. Osman, A.G.; Mekkawy, I.A.; Verreth, J. and Kirschbaum, F. (2007). Effects of lead nitrate on the activity of metabolic enzymes during early developmental stages of the Catfish ,*Clarias batrachus* (Burchell, 1822). Fish Physiol. Biochem., 33: 1-13.
11. Persch, T.S.P.; Weimer, R.N.; Freitas, B.S. and Oliveira, G.T. (2017). Metabolic parameters and oxidative balance in juvenile *Rhamdia quelen* exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. Chemosphere, 174: 98-109.
12. Qu, R.; Wang, X.; Wang, Z.; Wei, Z. and Wang, L. (2014). Metal accumulation and antioxidant defenses in the freshwater fish *Carassius auratus* in response to single and combined exposure to cadmium and hydroxylated multi-walled carbon nanotubes. J. Hazard. Mater., 275: 89-98.

13. Rubio, I.; Martinez-Madrid, M.; Méndez-Fernández, L.; Galarza, A. and Rodriguez, P. (2016). Heavy metal concentration in feathers of Little Egret (*Egretta garzetta*) nestlings in three coastal breeding colonies in Spain. Ecotoxicol., 25: 30-40.
14. Saliu, J.K. and Bawa-Allah, K.A. (2012). Toxicological effects of lead and zinc on the antioxidant enzyme activities of post juvenile *Clarias batrachus*. Resources and Environ., 2: 21-26.
15. Shafiq-ur-Rehman (2003). Lead-exposed increase in movement behavior and brain lipid peroxidation in fish. J. Environ. Sci. Health, Part A 38: 631-643.

Effects of lead on some oxidative stress of the Catfish, *Clarias batrachus*

1. Soegianto, A.; Moehammadi, N.; Irawan, B.; Affandi, M. and Hamami, M. (2010). Mercury concentrations in edible species harvested from Gresik coast, Indonesia and its health risk assessment. Cahiers de Biologie Marine, 51: 1.
2. Szebedinszky, C.; McGeer, J.C.; McDonald, D.G. and Wood, C.M. (2001). Effects of chronic Cd exposure via the diet or water on internal organ_specific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. and Chem., 20: 597-607.
3. Wu, M.; Xu, H.; Shen, Y.; Qiu, W. and Yang, M. (2011). Oxidative stress in zebrafish embryos induced by short_term exposure to bisphenol A, nonylphenol, and their mixture. Environ. Toxicol. and Chem., 30: 2335-2341.
4. Zikić, R.; Stajn, A.; Pavlović, S.; Ognjanović, B. and Saićić, Z. (2001). Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish *(Carassius auratus* gibelio Bloch.) exposed to cadmium. Physiol. Res., 50: 105